Performance of marking techniques in the field and laboratory for *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae)

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Abstract

A reliable marking technique was needed for a mark–release–recapture experiment with adults of *Diabrotica speciosa* (Germar). Four marking techniques, acrylic paint (spattered or brushed on the surface of the insect); and fluorescent pigments (dusted on surfaces or mixed with diet to produce an ingested marker), were tested. Fluorescent pigment durability for the dusting and ingested techniques was evaluated for laboratory conditions and under simulated field conditions. The impact of the techniques on beetle survival was also assessed. Both acrylic paint techniques caused mobility problems in the beetles, and neither technique lasted for more than 48 h. Both fluorescent pigment techniques were more reliable, but the dusting technique showed a significantly higher mortality than the control, and duration variations between laboratory and field conditions. Use of fluorescent pigments added to the diet was the most reliable technique. This technique allowed the manipulation of the marking period, and provided reliable timing of marker persistence in the field.

Introduction

Diabrotica speciosa (Germar) (Coleoptera: Chrysomelidae: Galerucinae) is the most widespread and abundant pest corn rootworm in South and Central America (Christensen 1943). It attacks at least 90 species of crops and ornamentals (Cabrera Walsh and Cabrera 2004). Control of diabroticine corn rootworms and cucumber beetles currently demands the use of a considerable amount of insecticide, often on a preventive basis, and with only temporary results (Sutter and Lance 1991). Of the alternative control methods that have been attempted, some of the most promising are cucurbitacin-based toxic baits; however, these have shown irregular results for yet unexplained reasons (Chandler 2003; Gerber et al. 2005). For most insects, cucurbitacins are plant-synthesized feeding inhibitors, yet they act as powerful attractants to several groups of leaf beetles. Previously, we evaluated the effect of cucurbitacin-rich extracts at different scales, from point source releases to broad 1-ha applications. This evaluation of insect movement relative to an applied treatment required a mark–release–recapture scheme, which demanded the evaluation of suitable marking techniques for *D. speciosa*. Of the usual methods that were considered and tried, we selected fluorescent pigment dusts and acrylic paint because they were economically and technically suited to our requirements (Hagler and Jackson 2001; Cronin 2003; Mo et al. 2003; Toepfer et al. 2005). For a detailed review of the techniques used to date to mark Diabroticina see Toepfer et al. 2005.

This work reports the results of several marking experiments that evaluated the easiest marking technique, the most reliable one for use in the release–recapture experiment, and their effect on beetle survival as compared with an unmarked control beetle population.

Materials and Methods

Four techniques for marking adult *D. speciosa* were studied: (1) Dots of ivory black (product code:

880 G-1), cobalt blue (844 G-3), titanium white (810 G-1) and cadmium red (834 G-4) acrylic colours (S.A. Alba, Garin, Argentina) were painted on the elytra with a 000 grade camel hair brush (Walker and Wineriter 1981; Hagler and Jackson 2001). The subject beetles, 10 for each treatment, were extracted from the laboratory colony maintained at the USDA-ARS South American Biological Control Laboratory with aspirators, immobilized at 5°C for painting, and afterwards transferred to 2-l cages with water tubes and beehive pollen as food source, and kept in a rearing chamber at 25 ± 1°C, on a 14 : 10 L/D photoperiod for observation. (2) Beetles, 10 for each colour, were spattered from paint-covered toothbrushes with fine droplets of the same acrylic colours, and transferred to cages in the same conditions for observation. (3) Fluorescent-powdered pigments, horizon blue (product code: A-19), corona magenta (A-21), fire orange new (A-14-N) and signal green (A-18-N) (Day-Glo, Cleveland, OH, USA), were dusted on the beetles by enclosing 50 beetles extracted from the laboratory colony with aspirators in a polyethylene bag with ca. 16 mg of pigment, and shaken gently for around 5 s. They were transferred from the bag to a clean plastic container where they could shake off excess powder. Finally, they were transferred to a 43-l cubical cage with water tubes with cotton wicks and beehive pollen as food source, and kept in a rearing chamber at 25 ± 1°C, and a 14:10 L/D photoperiod for observation. (4) The same fluorescent pigments were mixed with their standard meridic diet (Cabrera Walsh 2001) at 0.16% (w/w). The beetles were left to feed on the pigmented diet for 48 h, and transferred to cages in the same conditions for observation. The dusted beetles were distinguished from unmarked beetles by shining a 352/370 nm UV lamp on them (Sylvania 8 W F8T5/ BLB) in a dark room. The beetles fed the pigmented diet, however, had to be crushed on opaque, brown paper, or dissected under a microscope and then illuminated with the UV light in a dark room for discrimination.

With all the marking techniques, we tested for insect fitness modifications that might be attributable to marking technique due to an interference effect on movement, external receptors (Chapman 1998) and/or possible toxic effects of the marking substances. Consequently, all four marking techniques were evaluated in their marking endurance and effects on mobility. Both types of painted beetles were observed for 1 h immediately after painting, and again at 4, 24, and 48 h after marking, recording number of remaining marked beetles, dead beetles, and beetles with mobility defects. In the fluorescent pigment tests, marking duration was evaluated on colonies of 150 1-week-old beetles, one for each colour, by randomly removing with aspirators 15 beetles every day during the first 7 days, and once a week thereafter until the demise of the colonies. We recorded the number of marked beetles per sample. In every case, control beetles were kept next to the marked beetles in identical cages, under the same conditions as described above.

In addition, the four colours of powdered pigments were compared for duration in the ingestion marking system to test if the different colours had different permanence properties. In this test, four cages with 40 beetles/cage were each provided with a single coloured diet. Five beetles were removed every day with aspirators, and their gut was examined under a UV light to record the number of marked beetles per sample.

We recognized that the laboratory holding conditions did not approximate field conditions. The beetles were fed pollen, a highly nourishing food that could reduce ingestion and slow passage of the dye through the gut. Also, the small cages precluded flight, and smooth surfaces in them could aid the duration of the external marking. In a field situation, however, feeding on items with low nutritional value and high fibre contents, the brushing action of foliage, rinsing action of rain and damp foliage, and flight activity, could all reduce marker persistence. To better imitate a field situation, marking duration was also tested in outdoor 6-m^3 cubical field cages. We released 100 marked beetles in each cage, which contained one beehive pollen tray and a water tube, plus 15–50-cm tall maize plants in individual flowerpots and ca. 100 alfalfa seedlings in four elongated flowerpots arranged at random. Two cages had ingestion-marked beetles, and the other two had dusted beetles. The most and the least contrasting colours were used, corona magenta and signal green, respectively. Marking duration was evaluated by removing with aspirators and examining 10 randomly selected individuals under a UV light every 3 days, until no more marked beetles remained.

Mortality was evaluated for the beetles marked with fluorescent pigments in cages with 70 1-week-old beetles, by counting and removing the dead beetles every day –but weekends – for 2 weeks, with minimum disturbance. There were three treatments, dusted pigments, ingested pigments and controls, and each test was replicated twice. The beetles were kept in 43-l cages in a rearing chamber at 25 ± 1°C, and a 14 : 10 L/D photoperiod. The mortality data

for each treatment were pooled, and the mortality curves of both marking methods were compared individually against the control mortality curve with a Kaplan–Meier model. Significance levels were estimated with a log-rank Mantel–Haenszel test (SYSTAT Software, Inc. 2004).

Results

The acrylic paint dots method was rapidly discarded on the grounds that the paint did not adhere well to the waxy elytra of the beetles, chipping off easily in a few hours, such that by 48 h post-application almost no marked beetles remained (average = 1; SD = 0.82; n = 4 cages). It was also very time consuming for the projected number of beetles released in our tests, at around 33 min per 100 beetles. The marks applied with the spattering technique, while fast to implement, also chipped off easily. Spattering required a thorough coverage that also interfered with the beetles movements, and possibly sensilla as well. Specifically, beetles had problems opening their elytra to spread their wings, or suffered from stiff or stuck legs or antennae. After 48 h an average 65% mortality was observed (SD = 5.77; n = 4 cages).

Both the powder dusting and ingestion marking methods were effective at marking 100% of the subject beetles, at a very low cost and effort (0.01 US$ and ca. 50 min per 1000 beetles). However, the duration of the marking in the rearing chambers varied considerably between these techniques (fig. 1). Detection of ingestion-marked beetles declined after the fourth day, they were completely undetectable after 1 week. The dusting marking, on the other hand, lasted throughout the lifetime of the beetles (64 days, fig. 1), especially between the coxae and in other hairy ventral cavities. All four colours used in the discrimination test were clearly discernible under the UV light throughout the duration of the marking techniques.

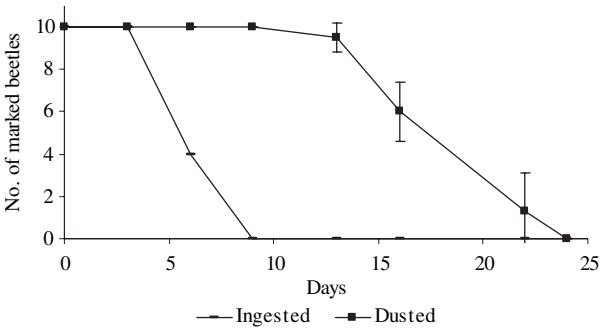

Fig. 2 Marking duration of the fluorescent pigment dusting and ingestion methods in field cages (average marked beetles ± SD).

The garden cage results were different from those in the rearing chamber for the dusting system, but they did not vary significantly for the ingestion method (fig. 2). The dusted beetles remained detectable for a median of 17.5 days, although the data range was quite large (13–22 days), indicating the pigment persistence was not consistently predictable after 10 days in the field cages. No persistence differences were detected between the two colours in either test.

Treatment mortality was not significantly different from the control in the ingestion test, but it increased significantly in the dusting test (χ^2 = 0.026, d.f. = 1, P = 0.872; and χ^2 = 10.799; d.f. = 1; P = 0.001, respectively). Within the timeframe of the

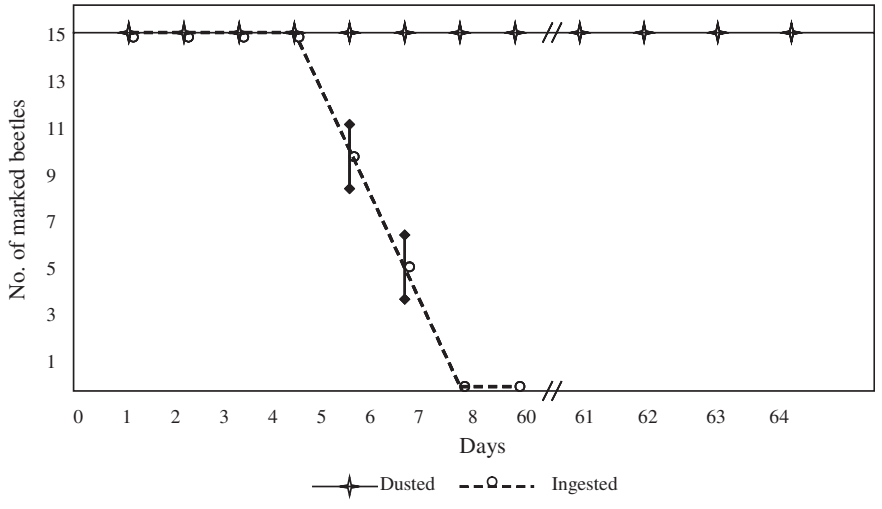

Fig. 1 Marking duration of the fluorescent pigment dusting and ingestion methods in a rearing chamber (average marked beetles ± SD). The x-axis is broken after 8 days, and resumed at 60 - days so that the slope of the ingestion test can be appreciated.

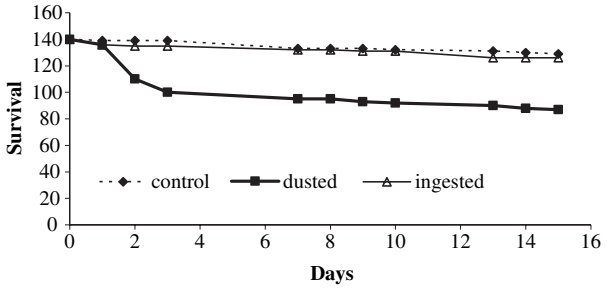

Fig. 3 Survival curves for the dusted and ingested pigment treatments and control beetles.

test, dusted beetles survived on average 4.01 days against 9.04 days for the diet pigmented and 9.4 days for the control beetles (fig. 3).

Discussion

There are at least three antecedents of marking Diabroticina by dusting with fluorescent pigments (Naranjo 1990; Oloumi-Sadeghi and Levine 1990; Toepfer et al. 2005). These publications did not present exactly comparable tests, but they all reported that no increased mortality was detected in the dusted beetles compared with the controls. We can only speculate on the factors underlying our lower survival of dusted beetles. The pigments we used were the same, or very similar, to those used in the other studies, as was the dusting technique. Moreover, we used between one-third and one-tenth of the doses of pigments used in the other works, so we have no reason to suspect toxic effects or spiracle obstruction not encountered by the other authors. Also, the number of beetles tested per colour in our tests was generally larger than in the previous works. The difference may reside in the robustness of the tests used in the other works, and the time lapse sampled. We compared the actual survival curves, while the other papers compared mortality after an arbitrary time lapse, which might mask initial survival differences as the subject colonies senesced. Also the time periods analysed were roughly between one-fourth and half the period covered by our survival tests. It is interesting to observe that the most important mortality was observed during the first days after dusting (fig. 3). This suggests that the deleterious effects of the pigments recede after much of it has fallen off.

Other reports of colour ingestion marking for Diabroticina (Lance and Elliott 1990; Naranjo 1990) used oil soluble dyes mixed with a dry diet. Interestingly, the duration of the marking was very similar to ours. However flight performance and survival were significantly affected by some batches of dye. Also, detection of the mark was rather unreliable even at pigment concentrations ca. 12 times higher than we used. The fluorescence of the pigments we used made marker detection under the UV light unequivocal.

Having rejected the acryl marking techniques, the choice between both fluorescent powder marking techniques should be matched to the objectives of the study. The dusting technique will last throughout the lifespan of the beetles under laboratory conditions. In the field, the evidence suggests that dusting will last longer than the ingestion method; however, its persistence is widely variable. Also, a certain increased mortality was observed under laboratory conditions. These mortality figures cannot be directly extrapolated to field conditions, but they suggest there could be an unpredictable risk of a fitness reduction for beetles in field release experiments. In addition, Naranjo (1990) reported that the flight pattern was slightly modified under a similar dusting marking system. Should a fitness reduction or behaviour modifications be critical in the development of an experiment involving insect marking, external dusting with fluorescent powders may not be a suitable choice.

In the case of our mark–recapture test, where several releases were done during the season, the ingestion-marking technique was best suited. As the recapture period spanned 96 h, this method guaranteed that the only marked beetles captured in traps would be those from the latest release. In addition, knowing the duration of effective marking allows greater control over release and recapture parameters. For example, knowing the beetles were effectively marked for 4 days after ingesting the dyed diet, their release could be delayed by a number of days to minimize the chances of overlapping recaptures with a different release.

One of the main conclusions of this work is that external dusting with fluorescent powders, a widespread and routine technique, probably persists for a shorter period under field conditions than in the laboratory conditions under which they are often devised and monitored. Another conclusion is that seemingly innocuous marking techniques, may turn out not to be so under every timeframe and analysis tool. Contrarily, the addition of small quantities of fluorescent pigments to an insect diet resulted in a stable marker presence and duration in both experiments, without detectable fitness

modifications, indicating it may be a reliable method within its time limitations.

Acknowledegments

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